PATENT
017191.0049

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re U.S. Patent Application		Confirmation No. 5494
Applicant:	Pugia et al.	Art Unit: 1644
Serial No.:	10/574,862	Examiner: Sharon Wen
Filed:	April 6, 2006	*I hereby certify that this correspondence is being deposited with the United Postal Service as Express Mail in an envelope addressed to: Mail Stop PCT, Commissioner of Patents, P. O. Box 1450, Alexandria, VA, 22313-1450, on* 2/26/08
For:	**Monoclonal Antibodies for Detection of Urinary Trypsin Inhibitors**	Alicia Eberhart *Signature*

AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This Amendment accompanies an RCE filed in response to a final Office Action dated October 3, 2007. Amendments to the specification are found on page 2. Amendments to the claims begin at page 5.

Please enter the following amendments to the specification.

Please insert the following on page 1 above **Background of the Invention**.

Priority is claimed from PCT/US2004/024881 filed July 29, 2004 which claims priority of U.S. provisional application No. 60/511,835 filed October 16, 2003.

Please replace line 4 of page 24 as follows: ~~Example 7~~ Example 8.

Please replace the paragraph beginning at page 6, line 19 and ending at line 28 with the following amended paragraph.

In another aspect, the invention includes methods of using the novel monoclonal antibodies to detect and measure forms of urinary trypsin inhibitors of interest, that is, those characterizing persons having disease. Where a monoclonal antibody is specific to an inhibitory UTI, e.g. to Bikunin, and Uristatin, or to the precursor AMBK, they can be measured directly. When a monoclonal antibody is able to bind to more than one UTI, then by using more than one antibody, the content of a particular UTI of interest may be determined by difference. The three monoclonal antibodies referred to above have been found to bind preferentially to Uristatin and Uristatin-1 and -2. ~~(Mab 421-5G8), to the Tamm-Horsfall protein (THP) (Mab 420-5D11), and to all UTIs and THP~~, but not to the pro-inhibitors ~~(421-3G5)~~.

Please replace the paragraph beginning at page 10, line 23 and ending at page 11, line 4 with the following amended paragraph.

BALB/c mice were immunized with 100 μg/mouse of purified UTIs obtained from renal patients by SciPac Ltd. Sittingbourne, Kent, UK, product code P250-1 to produce a response. ~~The immunogen composition as determined by SDS-PAGE was 15-20% 17kDa, 50-55% 35 kDa, and 25-30% 60-80 kDa with some material in the 2 to 12 kDa range~~. After one month, ocular bleeds were taken from each mouse and titered by ELISA against the immunogen to assess the immune response. The mice showing the best response were boosted by injection of

100 μg/mouse with immunogen. After four days, mice were sacrificed and their spleens used for fusion according to the method of Kohler and Milstein, Nature 256:495 (1975). The spleenocytes were fused with SP2-0 Ag14 myeloma cells using PEG (polyethylene glycol) solution with a ratio of spleenocytes to Myeloma cells of 5:1 and plated into 96 well plates using 50% PEG/HAT growth media. After 7-10 days of incubation at 37 degrees Celsius, fusion cultures were monitored for growth by feeding every 3-4 days utilizing the HAT (hypoxanthine, aminopterin, thymidine) selection method followed by subculturing with HAT growth media.

Please replace the paragraph beginning at page 15, line 15 and ending at page 15, line 23, with the following amended paragraph.

The monoclonal antibody 421-5G8 bound strongly to UTI lots #124-111 and #20-120 to a similar degree, but only bound weakly to Lot #80-117. This would be consistent with binding to the 2-12 kDa material (Uristatin 1 or 2) in both lots but not in lot 80-117 containing only the 17 kDa material (Uristatin). Thus, this antibody appears specific for Uristatin 1 or 2 over Bikunin or Uristatin. While not wanting to be limited to a mechanism, it is believed that binding by this monoclonal antibody does not occur through the sulfated chondroitin chain ~~as the moieties are known to possess high antigen affinities for antibodies~~. The binding is thought to be direct to an inhibitory amino acid sequence.

Please replace the paragraph beginning at page 15, line 24 and ending at page 16, line 8 with the following amended paragraph.

The monoclonal antibody 420-5D11 bound strongly to UTI lot #20-120, very weakly to lot #124-111, and did not bind to lot 80-117. This is consistent with binding to the 80 kDa material (THP) in lot #20-120, since only lot #20-120 contains a large amount of this material. If the antibody were specific for Uristatin or Bikunin also, one would expect it to have strongly responded to lot #124-111, which contained about 65% of Uristatin. This antibody appears to bind to THP but also to Bikunin and Uristatin. While not wanting to be limited to a mechanism, it is believed that binding by this monoclonal antibody does not occur through a common amino

acid region between THP and Bikunin or Uristatin. ~~Sequence matching has shown enough homology in the Uristatin 1 and 2 domains for this to be possible~~.